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A STUDY OF THE NEUTRALIZING PROPERTIES OF ANTIPOLIOMYELITIC HORSE SERUM

(NEUTRALIZATION OF VIRUS IN VITRO)

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The observation that a pleomorphic coccus is present in the central nervous system in poliomyelitis in man as well as in the experimental disease in monkeys, has been confirmed generally and apparently no longer is open to any serious question.

In contrast with the globoid bodies of Flexner and Noguchi,[1] which possess but feeble antigenic properties, it has been shown that intravenous injections of the poliomyelitic coccus in animals produce antibodies in high concentration[2] and that such serum possesses protective and curative properties[3] in experimental poliomyelitis of monkeys. The application of this knowledge to the treatment of poliomyelitis in man was made by Nuzum[4] who treated 10 cases of poliomyelitis with promising results during the latter part of the Chicago epidemic of 1916. Subsequently a total of 220 patients were treated with immune horse serum during the Chicago epidemic of 1917 and the favorable results[5] appear to coincide with those obtained by Rosenow[6] in a series of 58 cases occurring in Davenport, Iowa.

The apparently beneficial results from serum therapy, especially when used early in the preparalytic stage of the disease, and the harmlessness of the method when all due precautions are observed, justify a thorough and extensive trial of the serum. At the same time it is of the greatest importance to use only serum which possesses specific antibodies, if good results are to be obtained.

It is well known that recovery from poliomyelitis is accompanied by the appearance of neutralizing substances in the blood serum; this

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[1] Jour. Exper. Med., 1917, 25, p. 545.

[2] Nuzum, J. W.: Jour. Am. Med. Assn., 1917, 68, p. 24.

[3] Rosenow, E. C.: Jour. Am. Med. Assn., 1917, 69, p. 261. Nuzum, J. W.: Jour. Infect. Dis., 1918, 22, p. 258.

[4] Bull. Chicago Med. Soc., April 21, 1917.

[5] Nuzum, J. W., and Willy, R. G.: Jour. Am. Med. Assn., 1917, 69, p. 1247.

[6] Jour. Infect. Dis., 1918, 22, p. 379.

is the case in both man and monkey. The test of the potency of antipoliomyelitic horse serum, I believe, can be accurately determined in monkeys by the neutralizing properties of the serum against active virus in vitro. A study of this neutralization of virulent monkey virus by serum in vitro forms the basis of this paper.

EXPERIMENTS

A highly active monkey adapted virus was employed, which is now in its 25th generation and possesses the maximal degree of virulence for monkeys. I have followed the technic used by others in this field. A 5% suspension of fresh or recently glycerolated spinal cord and brain in normal salt solution was carefully prepared by grinding the tissues in a mortar with sterile sand and filtering 3 times through coarse filter paper to remove any gross particles of brain tissue. In other instances the suspension was passed through a Berkefeld filter and the clear filtrate employed in the tests. The immune horse serum was thoroughly mixed with the filtrate; for controls, similar mixtures of filtrate with normal horse serum and immune serum were used; the mixtures were placed in the incubator for 3 hours and in the ice-chest for 18-21 hours prior to inoculation into monkeys. In each series of experiments rhesus monkeys of approximately equal size were chosen. Each animal received the respective mixture of serum and virus intracerebrally under ether anesthesia. The protocols follow:

SERIES 1.

Exper. 1.—Monkey 63. (Control.) Dec. 5, 1917, 3 p. m.: Monkey etherized and given an intracerebral injection of 0.5 c c of a 5% suspension of fresh cord and brain of paralyzed Monkey 60.

Dec. 11: Onset of symptoms with marked tremor of the head. Climbs awkwardly in the cage.

Dec. 12: Flaccid paralysis of both legs.

Dec. 13: Severely paralyzed in all extremities.

Dec. 14: Prostrate in cage and in a dying condition. Typical lesions of poliomyelitis.

Exper. 2.—Monkey 64. (Control.) Dec. 5, 1917, 3:30 p. m.: Etherized and given an intracerebral injection of 0.3 c c of a 5% suspension of fresh Monkey Virus 60.

Dec. 12: Tremor of head and neck muscles. Animal quiet in cage.

Dec. 13: Complete flaccid paralysis of the legs. Marked tremor.

Dec. 14: Paralysis extending. Arms weak. Cannot sit up in the cage. This monkey survived the infection and ultimately regained good use of the arms. The hind legs remained flaccid and now exhibit marked atrophy and contractures.

Exper. 3.—Monkey 65. Dec. 5, 1917: 0.3 c c of Virus 60 plus 0.6 c c of immune horse serum were thoroughly mixed, placed in the thermostat for 3 hours and then in the ice-box for 21 hours. Monkey was etherized and given an intracerebral injection of this virus serum mixture.

Dec. 20: Monkey remains well and apparently normal.

Jan. 10: Has never exhibited any symptoms of poliomyelitis.

Exper. 4.—Monkey 66. (Control.) Dec. 5, 1917: 0.5 c c of virus plus 0.5 c c of normal horse serum were previously thoroughly mixed and incubated for 3 hours and placed in the ice-box for 21 hours. Monkey etherized and injected intracerebrally with this mixture.

Dec. 10: Monkey prostrate in cage. 10 p. m.: marked respiratory embarrassment. Death. Microscopic sections of the medulla and cervical cord revealed marked lesions of poliomyelitis.

Exper. 5.—Monkey 67. Dec. 5, 1917: Intracerebral injection of a virus serum mixture prepared as follows: 0.5 c c of Virus 60 plus 0.5 c c immune horse serum were mixed and incubated for 3 hours and placed in the ice-box for 21 hours.

Dec. 20: Monkey has remained well.

Dec. 26, 12 a. m.: Monkey found prostrate in cage with flaccid paralysis of legs and weakness of right arm.

Dec. 28: Severely paralyzed in all extremities. Death at 7 p. m. Typical cord lesions of poliomyelitis.

Exper. 6.—Monkey 68. Dec. 5, 1917: Etherized and given an intracerebral injection of a virus serum mixture prepared as follows: 0.5 c c of Virus 60 plus 0.5 c c fresh immune human serum were mixed in the test tube and placed in the thermostat for 3 hours and then in the ice-box for 21 hours.

Dec. 21, 9 a. m.—Monkey has a flaccid paralysis of both legs.

Dec. 23: Prostrate in cage.

Dec. 25: Died. Microscopic sections of the medulla and cord reveal typical lesions of poliomyelitis.

In the experiments in Series 1 the inactivated immune horse serum was obtained Nov. 11; preserved with 0.1% tricresol, passed through a Berkefeld filter and stored in the ice-chest 24 days prior to use. Three control monkeys, receiving virus alone or virus plus normal horse serum (Monkey 66), all developed poliomyelitis within periods of 5-7 days subsequent to inoculation. Monkey 65 was completely protected against the disease by the immune horse serum.

Monkeys 67 and 68 inoculated with virus plus immune horse serum and virus plus immune human serum exhibited a definite delay of 16 and 11 days, respectively, in the onset of the paralysis over that of the control Monkey 66. This observation is of especial interest since we have here a parallel between the antibody content of immune horse serum and the serum of a recently recovered human which is known to possess neutralizing properties against virus when the proper experimental conditions are fulfilled.

SERIES 2

In this series of tests the immune horse serum was obtained Jan. 8 and used fresh without preservative. All monkeys were injected with fresh 5% emulsion of cord and brain of paralyzed Monkey 18. The experiments were controlled both with virus alone and also with virus plus immune human serum known to possess neutralizing properties.

Exper. 7.—Monkey 69. (Control.) Jan. 9, 1918, 7 p. m.: Etherized and given an intracerebral injection of 0.2 c c of a 5% suspension of the cord from paralyzed Monkey 18.

Jan. 14: Onset of symptoms, namely, monkey huddled in corner of cage. Grinds teeth. Fine muscular twitchings.

Jan. 27: Return of previous symptoms. Monkey very excitable. Marked tremor. Weakness of left arm.

Jan. 28: Both legs and left arm flaccid. Monkey prostrate in cage.

Jan. 30: Died. Sections of medulla, cervical and lumbar cord revealed typical lesions of poliomyelitis.

Exper. 8.—Monkey 70. Jan. 9, 1918: Etherized and intracerebral injection of a virus serum mixture prepared as follows: 0.4 c c of Virus 18 plus 0.6 c c immune horse serum were carefully mixed and incubated for 2 hours and placed in the ice-box for 22 hours.

Feb. 15: Monkey remains well. Has never shown any symptoms of poliomyelitis.

Exper. 9.—Monkey 71. Jan. 9, 1918: Etherized and given an intracerebral injection of virus serum mixture prepared as follows: 0.3 c c of virus plus 0.3 c c immune horse serum were thoroughly mixed and incubated for 2 hours and placed in ice-chest for 22 hours.

Feb. 15: Monkey has remained entirely normal.

Exper. 10.—Monkey 72. Jan. 9, 1918: Intracerebral injection of a virus serum mixture composed of 0.5 c c of virus plus 1 c c immune horse serum previously incubated as previously described.

Feb. 15: Monkey has never exhibited any symptoms of poliomyelitis. Remains well.

Exper. 11.—Monkey 73. Jan. 9, 1918: Intracerebral injection of virus serum mixture prepared as follows: 0.3 c c of virus plus 0.6 c c immune horse serum were thoroughly mixed in test tube and incubated 2 hours and placed in ice-chest for 22 hours.

Feb. 15: Monkey remains well.

Exper. 12.—Monkey 74. (Control.) Jan. 9, 1918: Intracerebral injection of a virus serum mixture prepared as follows: 0.3 c c of virus plus 0.6 c c immune human serum were mixed and incubated 2 hours and placed in the ice-chest 22 hours before injection.

Feb. 15: Monkey remains entirely normal.

Exper. 13.—Monkey 75. (Control.) Jan. 9, 1918: Intracerebral injection of virus serum mixture prepared as follows: 0.4 c c of virus plus 1 c c immune human serum were mixed in a test tube and incubated 2 hours and placed in the ice-box 22 hours prior to injection.

Feb. 15: Perfect neutralization of the virus. The monkey remains well.

Exper. 14.—Monkey 76. Jan. 9, 1918: 0.4 c c of virus plus 1 c c of immune horse serum were thoroughly mixed, placed in the thermostat and in the ice-chest the usual time and injected intracerebrally under ether anesthesia into Monkey 76.

Feb 15: Monkey has remained well and has never shown any symptoms of poliomyelitis.

An analysis of this series of eight inoculations shows that the 5 animals receiving virus plus immune horse serum were completely protected against infection, even when 2½ fatal doses of virus were employed. Control Monkeys 74 and 75 inoculated with virus plus serum from convalescent poliomyelitic patients remain well, showing the technic of the experiments to be adequate for the neutralization of virus by serums known to possess such action. And finally Monkey 69 receiving but 0.2 c c virus developed typical poliomyelitis as demonstrated microscopically thereby proving the activity of the virus.

SERIES 3

For this series of inoculations all serums were reactivated with fresh guinea-pig complement in the proportion of 1 part of complement to 9 parts of serum. A 5% emulsion of recently glycerolated monkey viruses (Nos. 64 and 68) was prepared, filtered 3 times through coarse paper and employed in all these tests. The virus serum mixtures were in every instance thoroughly mixed and placed in the thermostat for 3 hours and in the ice-chest 21 hours prior to injection.

Exper. 15.—Monkey 77. (Control.) Jan. 19, 1918, 3 p. m.: Intracerebral injection of 0.5 c c of 5% virus suspension.

Jan. 23: Onset of symptoms, namely, excitability and tremor of head.

Jan. 25: Flaccid paralysis of both legs. Marked generalized muscular twitchings. Highly excitable.

Feb. 25: Monkey survived with a complete flaccid paralysis of the hind legs. Marked atrophy of legs.

Exper. 16.—Monkey 78. Jan. 19, 1918: Intracerebral injection of a virus serum mixture prepared as follows: 0.5 c c of above virus was mixed with 0.5 c c immune horse serum and incubated for 3 hours and placed in the ice-box for 24 hours before inoculation.

Feb. 25: Monkey has remained perfectly well.

Exper. 17.—Monkey 79. Jan. 19, 1918: 1 c c of immune horse serum was thoroughly mixed with 0.5 c c virus, incubated 3 hours and placed in ice-box over night. This mixture was injected intracerebrally under ether anesthesia.

Feb. 25: The monkey remains well.

Exper. 18.—Monkey 80. Jan. 19, 1918: A virus serum mixture consisting of 0.75 c c virus plus 1 c c immune horse serum was prepared and after the usual incubation period, the above mixture was injected intracerebrally.

Feb. 25: Monkey has never shown any symptoms of poliomyelitis.

Exper. 19.—Monkey 81. (Control.) Jan. 19, 1918: A large rhesus received an intracerebral injection of a virus serum mixture as follows: 1 c c of normal horse serum was thoroughly mixed with 1 c c of virus, reactivated with 0.2 c c guinea-pig complement and placed in the thermostat for 3 hours and in the ice-box 21 hours prior to injection.

Jan. 23: Monkey had a convulsion. Fur ruffled. Muscular tremor.

Jan. 25: Repeated convulsions. Legs flaccid. Arms spastic.

Jan. 26: Monkey died during a convulsion. Microscopically typical lesions of poliomyelitis in the medulla, pons and cord.

Exper. 20.—Monkey 82. Jan. 19, 1918: 1 c c of virus suspension was mixed with 1 c c immune horse serum, incubated 3 hours and placed in ice-chest 21 hours. The above virus serum mixture was then injected intracerebrally under ether anesthesia.

Feb. 25: Monkey has never shown the slightest symptoms of poliomyelitis and remains well.

Exper. 21.—Monkey 83. (Control.) Jan. 19, 1918: 1 c c of virus suspension was mixed with 1 c c of immune human serum, incubated 3 hours and allowed to stand in the ice-box over night. This mixture was injected intracerebrally into Monkey 83.

The four monkeys receiving immune horse serum plus virus were completely protected against infection. Normal horse serum does not possess this neutralizing power against virus as shown in the case of Monkey 81. Monkeys 77 and 83 serve to control these experiments both positively and negatively.

SERIES 4

In this last series of experiments an attempt was made to determine the maximal dose of virulent virus which can be neutralized by immune horse serum. A fresh 5% Berkefeld filtrate of the spinal cord of a prostrate paralyzed monkey was carefully prepared. The serum employed was obtained fresh the same day. The protocols follow:

Feb. 25: Perfect neutralization. The monkey remains entirely normal.

Exper. 22.—Monkey 84. (Control.) Feb. 9, 1918, 10 a. m.: Etherized and given an intracerebral injection of 0.5 c c of a 5% suspension of fresh virus.

Feb. 14: Onset of symptoms. Excitable.

Feb. 16: Monkey completely paralyzed in all extremities. Cannot raise head up from floor of cage.

Feb. 19: Etherized in dying condition. Marked typical lesions of poliomyelitis in the medulla, pons, cervical and lumbar levels of the spinal cord.

Exper. 23.—Monkey 85. Feb. 9, 1918: Intracerebral injection of a virus serum mixture prepared as follows: 1 c c of 5% emulsion of virus was thoroughly mixed with 1 c c of fresh immune horse serum and incubated for 3 hours and placed in the ice-box for 21 hours before injection.

March 20: Monkey remains well and has never had any symptoms of poliomyelitis.

Exper. 24.—Monkey 86. Feb. 9, 1918, 12 noon: 2 c c of a fresh 5% suspension of virulent virus was carefully mixed with 2 c c of fresh immune horse serum and placed in the thermostat for 3 hours and in the ice-box over night. This entire virus serum mixture totaling 4 c c was injected half into each cerebral hemisphere under ether anesthesia.

Feb. 14: Monkey appears excitable and races about his cage.

Feb. 15: Tremor of head and neck.

Feb. 16: Flaccid paralysis of both legs. Arms weak. Ptosis of right upper eyelid.

Feb. 28: Monkey has survived although so completely paralyzed as not to be able to raise his head from the floor.

March 20: Monkey still living with marked atrophy of all muscles of the trunk and extremities and beginning contractures.

It thus appears that immune horse serum neutralized 1 c c of a 5% suspension of virulent virus perfectly but could not neutralize 2 c c of this virus. That the virus was attenuated somewhat seems plausible since this monkey survived although so severely paralyzed as to be unable to take food alone.

DISCUSSION

A series of 24 monkeys were inoculated intracerebrally with virulent monkey adapted virus plus various serums after previous incubation of the virus serum mixtures for 2-3 hour periods followed by storage in the ice-box for 21 hours. Under the conditions of the experiment, immune human serum neutralized virus perfectly in 3 monkeys.

Immune horse serum protected 11 monkeys perfectly against the virus of poliomyelitis. In the 2 monkeys in which both immune human serum and immune horse serum failed to neutralize the virus, it appears noteworthy that the immune horse serum caused a delay of 16 days in the onset of paralysis as compared with a delay of but 11 days with the serum of a convalescent poliomyelitic patient which is known to possess neutralizing properties against the virus of this disease.

Fresh virulent monkey adapted virus of maximal potency was utilized in these experiments, recently glycerolated virus was employed in a few instances. The immune horse serum has been used both fresh and after storage for periods of 24 days in the ice-box, both with and without preservative and both activated and inactivated. The experiments were carefully controlled both positively and negatively. The 5 control monkeys receiving virus alone all developed typical symptoms of poliomyelitis even with virus doses of 0.2 c c of a 5% suspension thereby indicating the virulence of the virus employed.

The 2 monkeys receiving normal horse serum plus virus developed a typical, rapidly fatal poliomyelitis as proven microscopically. This is in accord with previous observations made by myself and others, i. e., normal horse serum does not possess the power to neutralize suitable doses of virulent virus but rather tends to shorten the incubation period of the disease. Finally the neutralizing properties of immune horse serum as demonstrated by these experiments would appear to justify the use of such a serum in the treatment of poliomyelitis in man.

SUMMARY

Antipoliomyelitic horse serum, prepared by repeated intravenous injections of the coccus isolated from the central nervous system in human and monkey poliomyelitis possesses neutralizing properties against the virus of poliomyelitis.

Immune horse serum protected 11 monkeys perfectly against fatal doses of virus. In 2 monkeys in which both immune human serum and immune horse serum failed to neutralize the virus, a definite delay of 16 days in the onset of paralysis must be attributed to the immune horse serum as compared to a shorter delay of 11 days with immune human serum known to possess much neutralizing principles.

The neutralizing properties of antipoliomyelitic horse serum in vitro for the virus of poliomyelitis afford a convenient and satisfactory test of the potency of this serum for use in the treatmnt of poliomyelitis. Suitable controls with normal horse serum and comparsions with immune human serums known to possess neutralizing properties should be conducted simultaneously.

Finally, the neutralizing, protective and curative properties of antipoliomyelitic horse serum for experimental poliomyelitis of monkeys are in direct accord with the favorable results observed in the serum treatment of human poliomyelitis and argue strongly for the etiologic relationship of the coccus to this disease.